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Efferent Mechanisms of Discharging Cnidae: II. A Nematocyst Release Response in the Sea Anemone Tentacle

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Abstract. Feeding behavior in cnidarians is a sequence of coordinated responses beginning with nematocyst discharge. The nematocyst response produces prey capture by envenomating prey and attaching prey to the tentacle. The strength of attachment of discharged nematocysts to the tentacle is termed intrinsic adherence and is calculated from measurements of adhesive force. Following prey capture, the feeding response involves movement of the tentacles toward the mouth and mouth opening. For ingestion to occur, nematocysts attaching the prey to the tentacles must be released from the tentacle. A nematocyst release response has been proposed, but never documented nor measured. Our criterion for a nematocyst release response is that the intrinsic adherence of discharged nematocysts must decrease to zero. The unit of nematocyst discharge in sea anemone tentacles is the cnidocyte/supporting cell complex (CSCC). The nematocyst response includes nematocysts discharged from Type C CSCCs by physical contact alone and nematocysts discharged from the more numerous Type B CSCCs that require both chemosensitization and physical contact. We identify two prey-derived substances, N-acetylneuraminic acid (NANA) and glycine, both of which chemosensitize nematocyst discharge from Type B CSCCs at low concentrations. At higher concentrations NANA stimulates the release response of Type Cs, and glycine stimulates the release response of Type Bs.

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Abbreviations: CSCC, cnidocyte/supporting cell complex; D-600, 2-methoxyverapamil; i_m, intrinsic adherence of nematocysts; NANA, N-acetylneuraminic acid; S_t, tentacle stickiness.

Introduction

Cnidarians are obligate predators. Prey captured by cnidarians are attached to tentacles by discharged nematocysts. The discharged nematocysts, with attached prey, must be released in order for ingestion to proceed (Ewer, 1947). It is not known how the discharged nematocysts are released from the tentacles, and it is not known whether such release is under physiological control. In this paper, we hypothesize a "nematocyst release response." Using methods we have developed to measure the strength of attachment of discharged nematocysts to the tentacles of sea anemones (Thorington and Hessinger, 1996), we experimentally test and measure the nematocyst release response.

We have coined the term "afferent mechanisms" of cnida discharge to refer to those processes acting *to* or *toward* the undischarged cnida to regulate or initiate discharge, and to distinguish them from mechanisms acting *out of* or *from* the discharged cnida's effector functions, which we have termed "efferent mechanisms" (Thorington and Hessinger, 1996). Cnidae are the eversible secretory products of specialized cells called cnidocytes. The three known classes of cnidae are nematocysts, spirocysts, and ptychocysts (Mariscal, 1974). We have defined and measured an efferent mechanism termed "tentacle adherence." Tentacle adherence indicates how tightly the tentacle holds the capsules of discharged cnidae and is a measure of how tightly targets, such as captured prey, are retained on the tentacles. The force required to remove an average discharged spirocyst or nematocyst from tentacles is the "intrinsic adherence." The intrinsic adherence is calculated from measurements of adhesive force and of the numbers of nematocyst discharged.

We measure adhesive force directly by using a sensitive force-transducer. The adhesive force is the applied force needed to separate a target from the tentacle (Thorington and Hessinger, 1988a). Specifically, adhesive force, as measured from sea anemone tentacles, is the sum of contributions arising from the stickiness of the tentacle mucus to the target (S_t) and the product of the number of cnidae (mastigophore nematocysts, n_m, and spirocysts, n_s) discharging onto the target and their intrinsic adherence (mastigophore nematocysts, i_m, and spirocysts, i_s) (Thorington and Hessinger, 1996):

$$\text{Adhesive force} = S_t + (n_m)(i_m) + (n_s)(i_s) \quad \text{(Equation 1)}$$

Adhesive force and intrinsic adherence may be expressed in units of micronewtons (μN) or in hybrid units of milligram-force (mgf), since adhesive force is measured without a significant acceleration component and, thereby, does not involve Newton's Second Law (Miller, 1959).

We measured the intrinsic adherence of discharged spirocysts (i_s) and of nematocysts (i_m) in the sea anemone *Aiptasia pallida* (Thorington and Hessinger, 1996) and found that the values of i_s are consistently very low relative to the values of i_m. We concluded that the values of i_s are too low to significantly contribute to the measurement of adhesive force or to participate in the retention of struggling prey on feeding tentacles. Thus, equation 1 may be simplified:

$$\text{Adhesive force} = S_t + (n_m)(i_m) \quad \text{(Equation 2)}$$

In anemone tentacles, each cnidocyte is surrounded by two or more supporting cells. The supporting cells possess chemoreceptors (Watson and Hessinger, 1988) and mechanoreceptors (Watson and Hessinger, 1991) that detect prey and trigger nematocyst discharge. The cnidocyte, surrounded as it is by two or more supporting cells, constitutes a cnidocyte/supporting cell complex (CSCC), which we contend is the functional and morphological unit for triggering nematocyst discharge in the sea anemone tentacle.

Two of the three known types of CSCC in sea anemone tentacles are germane to the present study: Types B and C (Thorington and Hessinger, 1990). Some of the CSCCs in anemone tentacles can be made to discharge by mechanical stimulation alone; others require conjoint mechanical and chemical stimulation. We term the former (CSCCs that respond to mechanical stimulation alone) Type C CSCCs; and the latter (which require both chemical and mechanical stimulation) Type B CSCCs. Sensitizing chemoreceptors for N-acetylated sugars (*e.g.*, N-acetylneuraminic acid, NANA) and for certain amino compounds (*e.g.*, glycine, alanine, and proline) have thus far been identified. Stimulation of the chemoreceptors predisposes contact-sensitive mechanoreceptors to respond to contact mechanical stimuli that trigger discharge (Thorington and Hessinger, 1988a; 1990). Type C CSCCs, which are present in lower numbers than Type Bs, do not require chemosensitization but discharge in response to mechanical contact alone.

In addition to proposing the nematocyst release response, we hypothesize that the response is controlled by prey-derived chemicals, as are nematocyst-mediated prey capture (Thorington and Hessinger, 1988b) and the subsequent feeding response (Lindsted, 1971; Lenhoff and Heagy, 1977). Together, our hypotheses predict that certain prey-derived chemicals will lower the intrinsic adherence of discharged nematocysts.

Using methoxyverapamil (D-600) to selectively inhibit discharge from Type Bs, we show that (i) NANA inhibits the intrinsic adherence of nematocysts discharged from Type C CSCCs and, therefore, controls the release response of Type Cs; and (ii) glycine inhibits the intrinsic adherence of nematocysts discharged from Type B CSCCs and, therefore, controls the release response of Type Bs. We conclude that a nematocyst release response exists in sea anemones and that it is controlled by prey-derived chemicals that also control prey capture.

Materials and Methods

Maintenance of sea anemones

Monoclonal sea anemones (*A. pallida*, Carolina strain) were maintained individually in glass finger bowls containing natural seawater at 24° ± 1°C as previously described (Hessinger and Hessinger, 1981; Thorington and Hessinger, 1988a). Briefly, anemones were fed daily with freshly hatched brine shrimp nauplii (*Artemia salina*) and washed 4–6 h after feeding (Hessinger and Hessinger, 1981). Anemones were maintained on a 12/12-h photoperiod using white fluorescent lights at an intensity of 5.5 klux (66 $\mu Es^{-1}m^{-2}$). Animals were starved for 72 h prior to experiments.

Experimental animals and test solutions

Filtered, natural seawater was obtained from Kerckoff Marine Laboratory of California Institute of Technology at Corona del Mar, California. Animals of same size were starved 72 h prior to experimentation and kept under constant fluorescent light at 4.5 klux (54 $\mu Es^{-1}m^{-2}$) during the last 48 h of starvation. Exposure to continuous light enhanced the uniformity of anemone behavior and cnidocyte responsiveness. Just prior to use, the animals were gently rinsed to remove soluble waste, and the medium was replaced with test solutions. Unless otherwise stated, animals were permitted to adapt to the change of medium for 10 min before cnidocyte responsiveness was mea-

sured. N-acetylneuraminic acid (NANA), glycine, and methoxyverapamil (D-600) were obtained from Sigma Chemical Co., St. Louis, Missouri. Solutions of D-600 were made up fresh on the day of the experiment and protected from light. All test solutions were prepared in artificial seawater (ASW) adjusted to pH 7.62 with 1 *N* HCl or NaOH. The ASW consisted of 423 m*M* NaCl, 10 m*M* KCl, 24 m*M* $MgCl_2$, 25 m*M* $MgSO_4$, 10 m*M* $CaCl_2$, and 1.2 m*M* $NaHCO_3$. Calcium-free artificial seawater (Ca-free ASW) was prepared with the same components as ASW except that calcium chloride was omitted, the NaCl concentration was increased to 438 m*M*, and EGTA (ethyleneglycoltetraacetic acid) was added to a final concentration of 1 m*M;* and then the final pH was adjusted to 7.62.

Assays of cnidocyte responsiveness

Physical contact of a tentacle with a gelatin-coated probe triggers discharge of local nematocysts and spirocysts, and adherence of the tentacle to the probe (Thorington and Hessinger, 1988b, 1990). Four parameters were measured to analyze nematocyst-mediated adhesive force: total adhesive force; number of discharged nematocysts; number of discharged spirocysts; and adhesive force in tentacles in which nematocyst and spirocyst discharge had been inhibited by pretreatment with formaldehyde to measure tentacle stickiness. The methods have been described in detail previously (Thorington and Hessinger, 1990). The number of cnidae on the probes is a direct measure of the number of cnidae discharged.

Measurement of adhesive force. Cnida-mediated adhesive force was measured as previously described (Thorington and Hessinger, 1988a). This technique involves using small gelatin-coated nylon beads of defined diameter attached to a strain gauge by means of a fine stainless steel shaft. The gel-coated bead is made to contact the distal third of a primary tentacle on an anemone in a finger bowl containing the test solution. The discharge of cnidae initiated by contact of the probe with the tentacle causes the tubules of everting cnidae to either adhere to or penetrate the gelatin surface. Withdrawing the probe from the tentacle causes the discharged cnidae to exert an opposing force on the probe; this force is measured with a gravimetrically calibrated force-transducer connected to a potentiometric recorder. The force necessary to separate the probe from the tentacle is called the adhesive force and is expressed in hybrid units of milligram-force (mgf). It is an aggregate measure of the "inherent" stickiness of the tentacle plus the nematocyst-mediated adhesive force.

Counting discharged nematocysts. After adhesive force measurements, the same probes are processed for counting nematocysts as detailed previously (Geibel *et al.*, 1988). Briefly, the gelatin coating of the probes is enzymatically digested to release the nematocysts of the discharged mastigophores. The highly refractive mastigophores, which are resistant to proteolysis, are then counted with an inverted microscope from the flat bottoms of microtiter wells.

Counting discharged spirocysts. To determine the number of discharged spirocysts, we used an indirect, solid-state enzyme-linked lectin sorbant assay (ELLSA), the details of which have been published (Thorington and Hessinger, 1990). In principle, the assay is based upon the high affinity of conjugated N-acetylated sugars to the everted tubules of discharged spirocysts on the surface of the test probes. The assay involves use of a microtiter-plate spectrophotometer for colorimetric determination of bound peroxidase activity after the sequential treatment of test probes with solutions of asialomucin and *Vicia villosa* lectin/peroxidase conjugate.

Collection and analysis of data

Individual animals were tested at each concentration of sensitizer. Twelve probes (one per tentacle) were used on each animal to determine adhesive force and to count discharged nematocysts or spirocysts. Daily experimental means were calculated from these experiments. Replicate experiments were carried out on three different days. Each data point represents the mean of the three daily experimental means, and the range bar represents the standard error of the mean.

Results

Selective discharge of cnidae from Type C cnidocyte/supporting cell complex

To test our hypotheses that a nematocyst release response exists and is under the control of prey-derived chemicals, we ask the research question: Do known chemosensitizers of nematocyst discharge lower, in a dose-dependent manner, the values of i_m for nematocysts discharged from either Type B or Type C CSCCs? To answer this question we proposed to determine the intrinsic adherence (i_m values) for nematocysts discharged from Type B and from Type C CSCCs as a function of chemosensitizer concentration. We selectively blocked chemosensitized discharge of nematocysts from Type B CSCCs by using 2-methoxyverapamil (D-600) (Thorington and Hessinger, 1992; Watson and Hessinger, 1994b), a diphenylalkylamine inhibitor of vertebrate L-type calcium channels (Spedding and Paoletti, 1992). With discharge from Type B CSCCs blocked, we directly determined the effect of chemosensitizers on the intrinsic adherence of nematocysts discharged from Type Cs. By subtracting the responses of the Type Cs from the combined responses

of Type B and Type C CSCCs (in the presence of chemosensitizer, but the absence of D-600), we calculated the intrinsic adherence of nematocysts from the Type Bs.

D-600 inhibits discharge from Type B CSCCs. D-600 potently and dose-dependently inhibits nematocyst discharge from NANA-sensitized Type Bs (Fig. 1; open circles). D-600 also inhibits glycine-sensitized nematocyst discharge (Fig. 1; open squares), but less potently and over a wider range of D-600 concentrations. In the absence of chemosensitizers, mechanical contact elicits discharge only from Type C CSCCs (Thorington and Hessinger, 1990). D-600 has no detectable effect on nematocyst discharge from Type Cs at any tested dose (Fig. 1; closed circles). The half-inhibitory doses (IC_{50}) for D-600 on NANA- and glycine-sensitized discharge is below 10^{-16} *M* for NANA and 10^{-15} *M* for glycine, and the minimal doses that maximally inhibit (IC_{100}) sensitized discharge are about 10^{-10} *M* for NANA and 10^{-6} M for glycine. Thus, D-600 blocks nematocyst discharge from Type B CSCCs, but not from Type Cs.

D-600 lowers tentacle stickiness (S_t). We tested the effects of D-600 on tentacle stickiness (Table 1) by incubating anemones at room temperature in ASW and in Ca-free ASW with and without D-600. After 20 min we added formalin to 10% for 5 min to measure S_t with adhesive force probes in the absence of any cnida discharge. The value of S_t is not altered by 10% formalin (Thorington and Hessinger, 1996). As expected, neither nematocysts nor spirocysts are discharged in 10% formalin (Table 1). The mean value of S_t in ASW is 36.6 ± 0.9 mgf (358.7 ± 8.8 μN; Table 1), which is in close agreement with the value of 34.6 ± 0.7 mgf (339.1 ± 6.9 μN) that we previously reported for similar experimental conditions (Thorington and Hessinger, 1996). The values of S_t are decreased 44% from ASW controls by 10^{-10} *M* D-600 in ASW ($P < 0.001$) and 49% from ASW controls by 10^{-8} *M* D-600 in ASW ($P < 0.005$). In Ca-free ASW, the mean value of S_t is 31.3 ± 0.9 mgf (306.7 ± 8.8 μN, a value 15% lower ($P < 0.02$) than in ASW containing 10 m*M* Ca^{2+}; the combination of Ca-free ASW and 10^{-10} *M* D-600 causes a decrease of 55% from ASW ($P < 0.001$; Table 1). Thus, D-600 significantly and dose-dependently lowers tentacle stickiness (S_t).

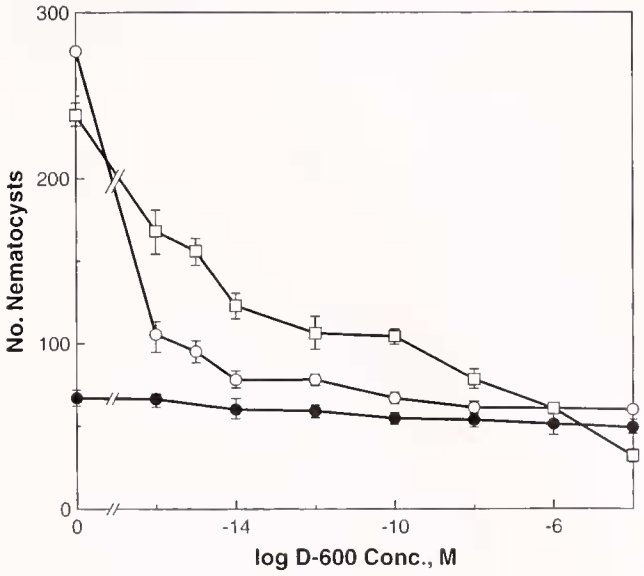

Figure 1. Effects of methoxyverapamil (D-600) on nematocyst discharge in the presence and absence of N-acetylneuraminic acid (NANA) or glycine. The number of nematocysts discharged from sea anemone tentacles onto probes at different molar concentrations of D-600 are indicated on the ordinate and abscissa, respectively, for anemones pre-exposed for 10 min to D-600 in seawater and then for 10 min more to D-600 with 1.8×10^{-5} *M* NANA in seawater (open circles; $n = 31$), or D-600 with 10^{-7} *M* glycine in seawater (open squares; $n = 13$), or D-600 in seawater (closed circles; $n = 33$). Data points are the mean ± standard error of the mean.

Effects of N-acetylneuraminic acid on intrinsic adherence

The combined dose-responses of Type B and Type C CSCCs to NANA for nematocyst discharge, spirocyst discharge, and adhesive force are characteristically biphasic (Fig. 2A–C, open circles; Thorington and Hessinger, 1990). In the presence of 10^{-10} *M* D-600, the dose-responses of Type Cs to NANA for nematocyst and spirocyst discharge are level and at control levels, showing no significant changes (Fig. 2A and B, closed circles). Thus, discharge from Type B CSCCs to NANA is totally inhibited by 10^{-10} *M* D-600, whereas discharge from Type Cs is unaffected.

NANA lowers i_m^c. Nematocyst-mediated adhesive force is calculated by subtracting tentacle stickiness (S_t) from adhesive force. To obtain nematocyst-mediated adhesive force in dose-responses to NANA, we subtracted from adhesive force measurements the appropriate value of S_t from Table 1 depending upon whether D-600 was or was not present during the experimental measurements. Nematocyst-mediated adhesive force from Type Cs steadily declines as the NANA concentration increases (Fig. 2C, closed circles). Since the numbers of nematocysts and spirocysts discharging from Type Cs do not significantly decrease (Fig. 2A and B, closed circles), the decline in adhesive force is probably due to a decrease in the value of i_m from the Type C CSCCs (*i.e.*, i_m^c).

We calculated the i_m values of nematocysts discharging from Type Cs (*i.e.*, i_m^c) across a wide range of NANA concentrations (10^{-16} to 10^{-3} *M*; Fig. 2D, closed circles) by using the values for nematocyst discharge and nematocyst-mediated adhesive force of Type C CSCCs in Equation 2. The values of i_m^c decrease with increasing levels of NANA, from about 0.20 mgf (1.96 μN) in ASW to

Table 1

Cnida-independent adhesive force (tentacle stickiness)

Treatment	Nematocysts[a] (n_m)	Spirocysts[b] (n_s)	Stickiness (mgf)[c] (S_t)
Pretreatment time = 90 min.			
ASW (without formalin)[d]	68.6 ± 0.7	136.7 ± 6.0	60.09 ± 1.00[e]
ASW[f]	0.30 ± 0.10	0.91 ± 0.35	36.55 ± 0.86
Ca-free ASW[g]	0.20 ± 0.12	3.45 ± 2.0	31.25 ± 0.88
Pretreatment time = 70 min.			
ASW + 10^{-10} *M* D-600[h]	0.22 ± 0.15	0.54 ± 0.14	20.44 ± 1.0
ASW + 10^{-8} *M* D-600[i]	0.31 ± 0.02	0.34 ± 0.25	18.49 ± 0.21
Ca-free ASW + D-600[j]	0.50 ± 0.28	0.30 ± 0.21	16.55 ± 2.0

Note: All anemones were pretreated with artificial seawater (ASW) at room temperature (23° ± 1°C) for either 70 or 90 min. If the treatment took place in Ca-free ASW, the pretreatment seawater was also Ca-free. After treatment, the anemones were contacted with test probes. Number of individuals (n) = 30 for all treatments except ASW without formalin, for which n = 34.

[a] Mean number of discharged nematocysts ± SEM.

[b] Mean number of discharged spirocysts ± SEM.

[c] Mean stickiness value measured as adhesive force, mgf ± SEM.

[d] Pretreatment only; no further treatment.

[e] Values include stickiness plus contributions from discharged nematocysts and spirocysts.

[f] Pretreatment + exposure to 10% formalin in ASW for 5 min.

[g] Pretreatment + exposure to 10% formalin in Ca-free ASW for 5 min.

[h] Pretreatment + exposure to ASW containing 10^{-10} *M* D-600 for 15 min, then exposure to 10% formalin in ASW for 5 min.

[i] Pretreatment + exposure to ASW containing 10^{-8} *M* D-600 for 15 min, then exposure to 10% formalin in ASW for 5 min.

[j] Pretreatment + exposure to Ca-free ASW containing 10^{-10} *M* D-600 for 15 min, then exposure to 10% formalin in Ca-free ASW for 5 min.

near zero at 10^{-4} *M* NANA. The concentration of NANA that half-inhibits i_m^c is approximately 10^{-12} *M* NANA. Thus, NANA dose-dependently lowers the intrinsic adherence of nematocysts discharged from Type Cs.

NANA biphasically modulates i_m^b. The dose-responses for discharge and adhesive force from Type Bs (Fig. 2A–C, closed squares) are calculated by subtracting the discharge (or adhesive force) of Type Cs in D-600 (closed circles) from the discharge (or adhesive force) of both Type Bs and Cs in the absence of D-600 (open circles). The calculated Type B dose-responses of both mastigophore and spirocyst discharge are narrow and biphasic. Maximum discharge (E_{max}) of nematocysts and spirocysts from Type Bs is about two times and one time, respectively, that of Type C ASW controls, suggesting that Type Bs and Type Cs coexist in the tentacle in ratios of 2:1 and 1:1 for nematocyst-bearing and spirocyst-bearing CSCCs, respectively. The concentration at which maximal discharge occurs (EC_{100}) is about 10^{-5} *M* NANA, and the concentration at which half-maximum discharge occurs ($K_{0.5}$) is about 10^{-6} *M* NANA. The NANA dose-response of nematocyst-mediated adhesive force from Type Bs is broadly biphasic, with an EC_{100} of 10^{-5} *M* NANA and a $K_{0.5}$ of about 10^{-10} *M* NANA (Fig. 2C, open circles).

Using the values for nematocyst discharge and nematocyst-mediated adhesive force of Type B CSCCs in Equation 2, we calculate the i_m values of nematocysts discharging from Type Bs (*i.e.*, i_m^b) across a wide range of NANA concentrations (Fig. 2D, closed squares). The i_m^b dose-response to NANA is biphasic, with maximum i_m^b values of about 0.8 mgf (7.8 μN) occurring at 10^{-10}*M* NANA and the $K_{0.5}$ occurring at about 10^{-12} *M* NANA. The peak of the i_m^b dose-response is about five orders of magnitude lower than the peak of the dose-response of Type B nematocyst discharge (Fig. 2A). We conclude that NANA dose-dependently modulates a biphasic change in the intrinsic adherence of nematocysts discharging from Type B CSCCs, but does not reduce the value of i_m^b to zero.

Effects of glycine on intrinsic adherence

Glycine suppresses discharge from Type Cs. The minimum dose of D-600 that totally inhibits glycine-sensitized nematocyst discharge from Type Bs is 10^{-6} *M* (Fig. 1). To minimize possible side effects from such a relatively high dose, we elected to use 10^{-8} *M* D-600 rather than 10^{-6} *M*. The dose-responses to glycine for nematocyst discharge, spirocyst discharge, and adhesive force are characteristically biphasic (Fig. 3A–C; Thorington and Hessinger, 1990). In 10^{-8} *M* D-600 the dose-responses to glycine for discharge of both nematocysts and spirocysts and for adhesive force do not exceed those of controls (Fig. 3A–C, closed circles). Thus, 10^{-8} *M* D-600 appears to inhibit cnida discharge from Type B CSCCs in response to glycine.

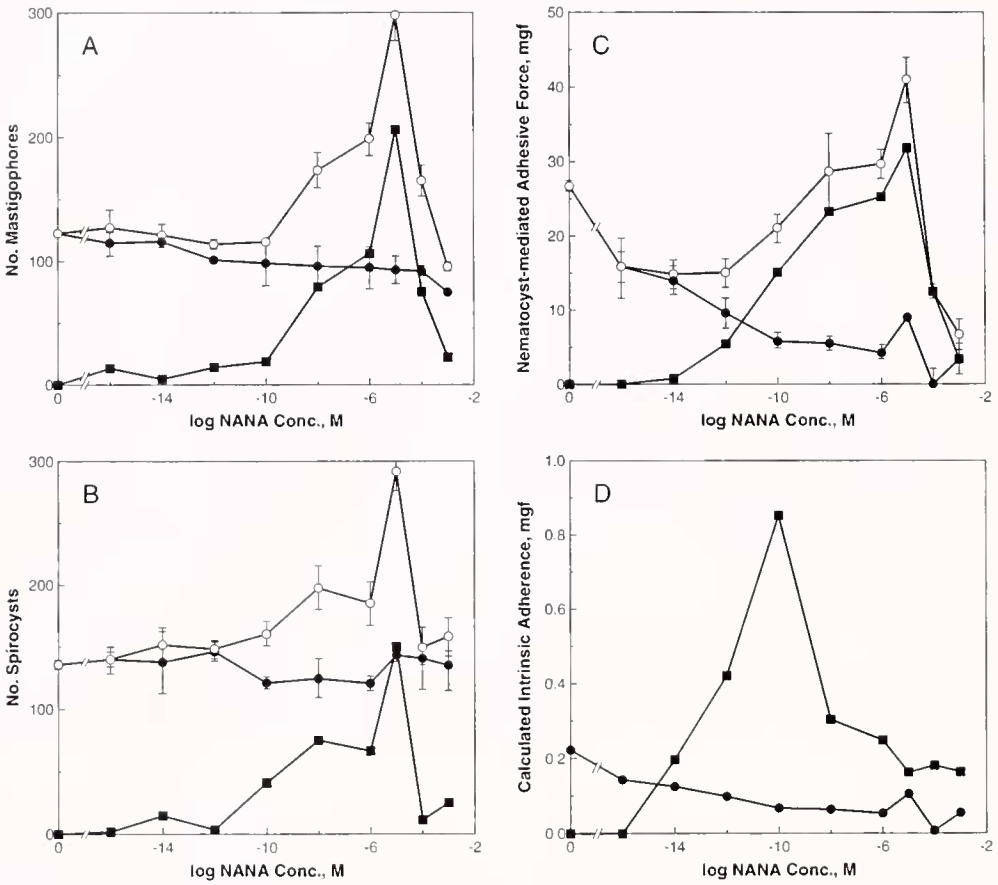

Figure 2. Afferent and efferent cnida dose-responses to N-acetylneuraminic acid (NANA). Combined cnida responses from Type B plus Type C cnidocyte/supporting cell complexes (CSCCs) are measured in seawater (open circles). Cnida responses from Type Cs only are measured in the presence of 10^{-10} *M* D-600 (closed circles). Calculated responses from Type Bs (closed squares) are obtained by subtracting Type C responses from combined Type B plus Type C responses. Values express the mean of three experiments. Each experiment consists of replicate probes for each NANA concentration; each probe and each tentacle is used only once. Vertical bars represent standard errors of the mean and are applied to all measured means, but not to calculated means. (A) Effect of NANA on nematocyst discharge (n = 34). (B) Effect of NANA on spirocyst discharge (n = 35). (C) Effect of NANA on nematocyst-mediated adhesive force (n = 36). (D) Effect of NANA on calculated intrinsic adherence.

On the other hand, in the presence of 10^{-8} *M* D-600, we observe about a 50% decrease in both nematocyst and spirocyst discharge from Type Cs at the lowest tested doses of glycine (*i.e.*, 10^{-16} *M* glycine). Nematocyst discharge gradually recovers to control levels of discharge at about 10^{-7} *M* glycine and then declines again at higher concentrations. Spirocyst discharge recovers at lower concentrations of glycine and reaches control levels at 10^{-6} *M* glycine before it, like nematocyst discharge, declines again at higher concentrations. Thus, low concentrations of glycine suppress both nematocyst and spirocyst discharge from Type C CSCCs.

Glycine modulates i_m^c. Nematocyst-mediated adhesive force from Type Cs remains constant (between 12 and 15 mgf or 118 and 147 μN) at all tested glycine concentrations except 10^{-7} *M* glycine, at which concentration the adhesive force increases to 20 mgf (196 μN; Fig. 3C; closed circles). Using Equation 2, we calculated the i_m values of nematocysts discharging from Type Cs (*i.e.*, i_m^c) across the range of tested glycine concentrations (10^{-16} to 10^{-4} *M*; Fig. 3D, closed circles). The values of i_m^c are relatively high (about 0.6 mgf/nematocyst or 5.9 μN/nematocyst) in seawater controls and in 10^{-16} to 10^{-12} *M* glycine. But between 10^{-10} and 10^{-7} *M* glycine, the values of i_m^c decrease by about one-half (to about 0.3 mgf/nematocyst or 2.9 μN/nematocyst) and then increase (to about 0.4 mgf/nematocyst or 3.9 μN/nematocyst) at 10^{-6} *M* and higher concentrations. Overall, there appears to be a

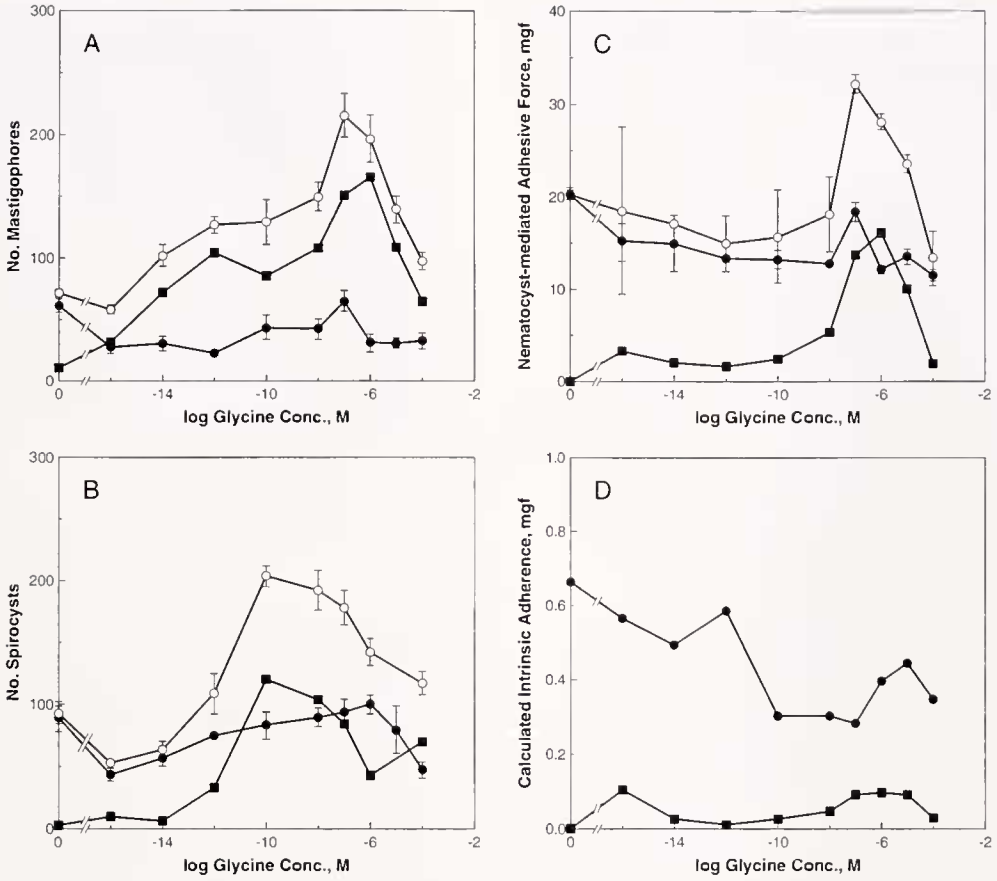

Figure 3. Afferent and efferent cnida dose-responses to glycine. Combined responses from Type B plus Type C cnidocyte/supporting cell complexes (CSCCs) are measured in seawater (open circles). Cnida responses from Type Cs only are measured in the presence of 10^{-8} *M* D-600 in seawater (closed circles). Calculated responses from Type Bs (closed squares) are obtained by subtracting Type C responses from combined Type B plus Type C responses. Values express the mean of duplicate experiments. Each experiment consists of replicate probes for each glycine concentration; each probe and each tentacle is used only once. Vertical bars represent standard error of the mean and are applied to all measured means, but not to calculated means. (A) Effect of glycine on nematocyst discharge (n = 15). (B) Effect of glycine on spirocyst discharge (n = 15). (C) Effect of glycine on nematocyst-mediated adhesive force (n = 14). (D) Effect of glycine on calculated intrinsic adherence.

downward trend in i_m^c with increasing glycine concentrations.

Glycine suppresses i_m^b. In contrast to NANA, in which NANA-sensitized discharge of nematocysts from Type Bs spans a narrow range of NANA concentrations (Fig. 2A, closed squares), glycine-sensitized discharge of nematocysts spans a wide range of glycine concentrations (Fig. 3A, closed squares). At all tested glycine concentrations (except 10^{-16} *M*), the numbers of nematocysts discharged from Type Bs exceed those discharged from Type Cs. Yet the values of i_m^b in glycine never exceed 0.1 mgf/nematocyst (0.98 μN/nematocyst; Fig. 3D, closed squares), unlike the values in NANA which never drop below 0.2 mgf/nematocyst (1.96 μN/nematocyst; Fig. 2D, closed squares).

Discussion

Cnidarians capture swimming prey by discharging nematocysts from their tentacles. These same nematocysts also secure the struggling prey to the tentacle during coordinated tentacle movements that transport the prey to the oral disk and mouth. For ingestion to occur, however, the nematocysts securing captured prey to the tentacle must then be released from the tentacle (Ewer, 1947).

We proposed a so-called nematocyst release response controlled by soluble, prey-derived chemicals also known to both chemosensitize nematocyst discharge and initiate the feeding response. We stipulated that the

strength of attachment of discharged nematocysts to sea anemone tentacles (*i.e.*, intrinsic adherence) must be reduced to or near zero for the mechanism to qualify as a nematocyst release response.

Effects of N-acetylneuraminic acid and glycine on discharge and intrinsic adherence

NANA does not affect discharge from Type Cs. Because low concentrations of D-600 block both NANA-sensitized and glycine-sensitized discharge from Type B CSCCs without affecting discharge from Type Cs (Figs. 1 and 2A, B), we were able to measure the response from Type Cs without interfering contributions from discharging Type Bs. Throughout the range of 10^{-16} to 10^{-3} *M* NANA, nematocyst and spirocyst discharge from Type Cs was constant (Fig. 2A, B). We concluded that neither nematocyst nor spirocyst discharge from Type Cs is affected by NANA chemoreceptor stimulation.

NANA controls the release response of Type Cs. Despite constant nematocyst discharge from Type Cs at all tested NANA concentrations, nematocyst-mediated adhesive force from Type Cs declined with increasing NANA concentrations (Fig. 2C). The decline in adhesive force was caused by a dose-dependent decrease (from 0.2 to 0 mgf/nematocyst, or 1.96 to 0 μN/nematocyst; Fig. 2D) in the intrinsic adherence of nematocysts discharged from Type Cs (i_m^c). The dose-dependent decrease in i_m^c fits our criterion of a nematocyst release response. We concluded that a release response of nematocysts discharged from Type Cs is under the control of NANA chemoreceptors.

NANA controls i_m^b. We calculated the NANA dose-responses of Type B CSCCs by subtracting NANA dose-responses in the presence of 10^{-10} *M* D-600 from NANA dose-responses in the absence of D-600 (Fig. 2). The calculated NANA dose-responses of both nematocyst and spirocyst discharge from Type Bs were narrow and biphasic, with maximal discharge occurring at 10^{-5} *M* NANA (EC_{100}) and half-maximal discharge ($K_{0.5}$) occurring at 10^{-6} *M* NANA (Fig. 2A, B). The calculated NANA dose-response of nematocyst-mediated adhesive force from Type Bs was also biphasic, but over a much broader range of NANA concentrations, with the EC_{100} still occurring at 10^{-5} *M* NANA, but with the $K_{0.5}$ occurring much lower (at 10^{-10} *M* NANA; Fig. 2C).

In NANA, the intrinsic adherence of nematocysts discharged from Type Bs (i_m^b) varied biphasically with NANA concentration (Fig. 2D). The maximal i_m^b value of 0.8 mgf/nematocyst (7.8 μN/nematocyst) occurred at 10^{-10} *M* NANA, but decreased to about 0.2 mgf (1.96 μN) at 10^{-5} *M* NANA and at higher concentrations. The values of i_m^c in NANA varied from 0.2 to 0 mgf/nematocyst (1.96 to 0 μN/nematocyst). Thus, in seawater containing NANA, i_m^b will exceed i_m^c. It therefore appears that in the presence of NANA, nematocysts discharged from Type Bs will predominate in both prey capture and prey retention over nematocysts discharged from Type Cs. Although the NANA dose-response of i_m^b never goes to zero, we conclude that NANA controls i_m^b over a wide range of values, in a dose-dependent manner, but without affecting the release response of nematocysts discharged from Type Bs.

Glycine inhibits discharge from Type Cs. In addition to N-acetylated sugars, amino sensitizers, such as glycine, also predispose nematocysts and spirocysts to discharge in response to mechanical stimuli (Thorington and Hessinger, 1988a, 1990). In the present study, we showed that glycine sensitizes Type B CSCCs to discharge nematocysts and spirocysts (Fig. 3A, B). However, unlike NANA, which had no effect on discharge from Type Cs, glycine suppressed nematocyst and spirocyst discharge from Type Cs (Fig. 3A, B). The inhibitory effect of glycine was partial and occurred at both low and high concentrations, but reversed at intermediate concentrations. The inhibitory effect of glycine on discharge from Type Cs is probably different from the inhibitory effects of certain homogenate fractions of *Artemia* on prey capture in *Hydra* (Grosvenor and Kass-Simon, 1987) since the inhibitory action of glycine in *Aiptasia* is restricted to Type Cs and the net effect of glycine is to increase nematocyst discharge due to its sensitizing effect on the more numerous Type Bs (Fig. 3A). To the best of our knowledge, this is the first report of an identified chemoreceptor inhibiting nematocyst discharge.

The value of i_m^c varied from 0.6 mgf/nematocyst (5.9 μN/nematocyst; in seawater and in lower glycine concentrations) to about 0.3 and 0.4 mgf/nematocyst (2.9 and 3.9 μN/nematocyst; at higher glycine concentrations). Although NANA lowers the i_m^c to zero at higher concentrations, thereby constituting a nematocyst release response, glycine also lowers i_m^c, but without bringing the value to zero. Glycine inhibits discharge from Type Cs, but it does not affect the Type C nematocyst release response.

Glycine controls the release response of Type Bs. Although glycine-sensitized nematocyst discharge from Type Bs is robust at all concentrations greater than 10^{-14} *M*, the value of i_m^b is consistently low, never exceeding values of 0.1 mgf/nematocyst and reaching 0 mgf/nematocyst (0.98 and 0 μN/nematocyst) at 10^{-4} *M* glycine. We conclude that the action of glycine on the intrinsic adherence of nematocysts discharged from Type B CSCCs is consistent with glycine control of the release response of nematocysts discharged from Type Bs.

D-600 affects Type Bs differently than Type Cs. The inhibition of nematocyst discharge from Type B CSCCs by verapamil and other organic and inorganic inhibitors of voltage-gated calcium channels was first reported in the anemone *Haliplanella luciae* (Watson and Hessinger,

1994a). We now show that D-600, a water-soluble derivative of verapamil, inhibits both nematocyst and spirocyst discharge from Type Bs in *Aiptasia pallida* (Fig. 2A, B). The effects on nematocyst discharge of calcium depletion and of standard organic and inorganic calcium channel blockers (Watson and Hessinger, 1994a) are consistent with the involvement of calcium channels, pharmacologically resembling L-type calcium channels, in the chemosensory signaling pathway of Type B CSCCs. Our finding that NANA-sensitized discharge of nematocysts is much more sensitive to D-600 than is glycine-sensitized discharge (Fig. 1) suggests that the NANA and glycine chemosensory pathways utilize calcium in different ways.

Both D-600 and calcium-free seawater reduce tentacle stickiness, and these effects are additive (Table 1), suggesting common modes of action. Tentacle stickiness is mediated by the interaction of the mucus layer covering the tentacle surface with the probe. Tentacle mucus originates, in part, by a constitutive secretory process of apical secretory cells. Under conditions of injury or stress, the amount of surface mucus increases. Thus, tentacle mucus may also be contributed by regulated secretion. The nematocyst is a secretory product of the cnidocyte (Slautterback, 1961; Skaer, 1973). Exocytotic processes (*i.e.*, mucus secretion and nematocyst discharge [Holstein and Tardent, 1984]), in general, require extracellular calcium and calcium influx mediated by calcium channels (Bennett *et al.*, 1979). We hypothesize that D-600 and calcium-free seawater reduce tentacle stickiness and inhibit discharge from Type B CSCCs by affecting involved calcium channels.

Roles of N-acetylneuraminic acid and glycine in prey capture and prey ingestion

Prey capture. In our view the "NANA" receptor is the primary chemoreceptor for capturing prey since N-acetylated sugars are ubiquitous constituents of the surfaces of most aquatic prey. On the other hand, the activators of the "amino" chemoreceptors (glycine, alanine, and proline) occur in high concentrations in the hemolymph of crustacean prey (Gilles, 1979) and of *Artemia* nauplii (Clegg and Conte, 1980), the typical prey of laboratory-reared cnidarians. Presumably the amino sensitizers leak into the ambient medium from wounds inflicted by NANA-sensitized nematocysts, as originally suggested by Loomis of the glutathione-effected feeding response of *Hydra* (Loomis, 1955) and later confirmed by Lenhoff (1961). Proline, for instance, potently and negatively modulates NANA-induced tuning of vibration-sensitive mechanoreceptors associated with Type A CSCCs in a related anemone, *Haliplanella luciae* (Watson and Hessinger, 1994b). Alanine inhibits NANA-sensitized discharge from Type Bs in *Aiptasia* (unpubl. findings). And glycine inhibits discharge of nematocysts and spirocysts from Type C CSCCs (Fig. 3A, B). Thus, we view the "amino" receptors to be secondary chemoreceptors in prey capture, involved in negatively modulating the primary prey capture response to N-acetylated sugars. As secondary chemoreceptors, the amino receptors respond to chemicals leaking from wounded prey and assure that only the minimum adequate number of nematocysts are discharged. By conserving nematocysts and avoiding "overkill," the anemone devotes fewer prey nutrients to replacement of nematocysts and more to growth and reproduction.

Prey ingestion. Following prey capture and transport to the mouth, glycine from wounded prey acts in concert with NANA to stimulate the release responses of discharged nematocysts so that prey can be ingested. Our present work shows that NANA controls the release response of nematocysts discharged from Type Cs (Fig. 2D), whereas glycine controls the release response of nematocysts from the more numerous Type Bs (Fig. 3D).

It has been observed, although not, to our knowledge, published, that many cnidarians do not ingest dead prey, even though the dead prey elicit nematocyst discharge and adhere to tentacles (*e.g.*, pers. obs. by C. Hand, UC, Davis; K. Heidelberg, U. Maryland; H. M. Lenhoff, UC, Irvine). Discrimination between living and dead prey appears to occur after prey capture and may involve the detection of amino substances leaking from wounded prey. On the one hand, the very short timescale for a successful nematocyst response dictates that chemical discrimination in prey capture be cursory and based upon a single chemical cue, the external N-acetylated sugars. These sugars are common to living and dead prey, so discrimination is sacrificed for response speed and the drifting remains of dead prey containing no nutrients are occasionally captured. On the other hand, the subsequent release response and prey ingestion take place on a much longer timescale than prey capture. The release response is based upon dual chemosensory cues, N-acetylated sugars and glycine. Dead and nutrient-depleted prey, if captured, will not provide the amino chemosensory cue needed to stimulate a complete nematocyst release response, and such prey will not be ingested. The dual chemoreceptor requirement for the release response ensures that captured dead prey are not ingested and digestive resources are not committed to nutrient-poor prey.

The i_m-discharge plot explained

We showed previously (Thorington and Hessinger, 1996) that a plot of the weighted average i_m values *versus* the total number of discharged nematocysts (the so-called i_m-discharge plot) was inversely hyperbolic in shape for NANA and several amino chemosensitizers. Except for

the steepness of the i_m-discharge plot, our dilution/recruitment model, in general, simulated the plot. The dilution/recruitment model assumed that (i) the i_m values of mastigophore nematocysts from both Type B and Type C CSCCs are constant, (ii) the value of i_m^c is larger than the value of i_m^b, and (iii) the number of nematocysts discharged from Type C CSCCs is constant, (iv) while the number discharged from Type B CSCCs varies biphasically with increasing concentrations of chemosensitizer. In this model the discharge of a constant number of high-i_m nematocysts from Type C CSCCs is numerically diluted by the discharge of progressively greater numbers of lower-i_m nematocysts recruited by chemosensitization of Type B CSCCs.

Our present findings indicate that the recruitment/dilution model is incomplete in several ways. Firstly, the i_m values from Type Bs and Cs are not constant. We have shown that NANA biphasically varies i_m^b and glycine monophasically varies i_m^c. Secondly, the values of i_m^c are not always larger than the values of i_m^b. We have found that i_m^b is greater than i_m^c in the presence of NANA. Thirdly, the number of nematocysts discharged from Type C CSCCs is not always constant. In the presence of glycine the number of nematocysts discharging from Type Cs decreases. On the positive side, our assumption that the numbers of nematocysts discharged from Type B CSCCs varies biphasically with increasing concentrations of chemosensitizer was confirmed for both NANA and glycine.

Conclusions

1. Nematocysts perform at least three feeding-related functions. (i) Discharging nematocysts subdue swimming prey by penetrating and envenomating prey. (ii) Discharged nematocysts secure struggling prey to the tentacles by a combination of barbed nematocyst tubules fastened into the integument of prey and the initially high intrinsic adherence of nematocyst capsules' attachment to the tentacle. (iii) Discharged nematocysts subsequently facilitate prey ingestion by being released from the tentacle as a result of a large decrease in intrinsic adherence.

2. Each of the feeding-related functions of nematocysts is under the control of at least two chemoreceptor systems. One system is for N-acetylated sugars, such as N-acetylneuraminic acid (NANA), which occur on the surface of prey; the other is for "amino" substances, such as glycine, which occur in the blood of prey and are leaked into the medium from nematocyst-inflicted wounds.

3. The "NANA" receptor is the primary chemoreceptor controlling prey capture, while the "amino" receptors act to negatively modulate nematocyst discharge. Glycine, for instance, inhibits the discharge of nematocysts (and spirocysts) from Type C CSCCs over a wide range of concentrations.

4. To ensure that captured prey are tightly secured to the tentacle, the "NANA" receptor raises the intrinsic adherence of nematocysts discharged from Type B CSCCs at low concentrations of NANA (about 10^{-10} *M*).

5. To ensure that captured prey are subsequently ingested, the nematocyst release response is activated for nematocysts discharged from Type C CSCCs and from Type B CSCCs by higher concentrations (10^{-4} *M*) of NANA and glycine, respectively.

6. Cnida-independent tentacle stickiness, presumably due to surface mucus, is dose-dependently decreased by D-600 and by removal of extracellular calcium. The inhibitory actions of D-600 and low calcium on tentacle stickiness are additive.

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